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SOME FACTORS AFFECTING VERTICAL DISTRIBUTION AND RESISTANCE TO DESICCATION IN THE LIMPET, *ACMAEA TESTUDINALIS* (MÜLLER)[1]

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The vertical distribution of many organisms in the intertidal zone is governed by their ability to tolerate the stresses of exposure (Stephenson and Stephenson, 1949; Wilbur and Yonge, 1964). Desiccation, one of these stresses, is a dominant factor influencing distribution (Stephenson, 1942). An organism inhabiting higher levels of the intertidal zone must tolerate longer periods of desiccation than an organism inhabiting lower levels.

Broekhuysen (1940) investigated the role of desiccation in controlling the vertical distribution of six species of intertidal gastropods of the genera *Littorina, Thais, Oxystele,* and *Cominella.* Results of his laboratory experiments indicated that species inhabiting higher levels tolerated greater body water loss before death than species inhabiting lower levels. He concluded that species from higher levels were least sensitive to desiccation. Davies (1969) studied limits of tolerance to desiccation in the limpet *Patella vulgata.* He found that high intertidal specimens of *P. vulgata* were tolerant of a greater loss of body water than low intertidal specimens. He concluded that, within a species, specimens from higher levels were more tolerant of desiccation.

Other investigators have shown the same interspecific and intraspecific variation in tolerance of desiccation between organisms found high and low in the intertidal zone (Colgan, 1910; Gowanloch and Hayes, 1926; Allanson, 1958; Brown, 1960; Davies, 1965).

Intertidal organisms exhibiting a high tolerance of desiccation possess behavioral, physiological or morphological characteristics which are adaptive. In limpets, size and shape of the shell are characteristics which have been correlated with tolerance of desiccation. In *P. vulgata,* Orton (1932) observed that steeper shells were predominant among those from lower levels. Davies (1969) observed the same phenomenon in *P. vulgata.* He stated that steeper shells were adaptive because the smaller circumference per unit volume reduced the amount of water lost between the edge of the shell and the substrate.

Body size has also been related to tolerance of desiccation in limpets. Lewis (1954), Frank (1965), Glynn (1965), and Blackmore (1969) found that large limpets were most numerous at upper levels of the intertidal zone and small limpets were most numerous at lower levels. Davies (1969) determined that rate of body water loss varied inversely with size in *P. vulgata.* Larger limpets could

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inhabit upper intertidal levels because they lost body water less rapidly. Shotwell (1950) however, found smaller limpets more numerous in the upper intertidal levels, with large individuals restricted to lower levels. He attributed this to relatively greater storage space for water between the shell and mantle in small limpets.

The size distribution of limpets in the intertidal zone is not always correlated with tolerance of desiccation. Sutherland's (1970) study of the population dynamics of *Acmaea scabra* showed that lower population density of limpets in the upper zone resulted in a more rapid growth rate and a population of larger limpets. In the lower zone, however, high population density restricted growth and the limpets were smaller. In addition to promoting rapid growth, the lower density of the upper zone resulted in a lower mortality rate than the lower zone.

The vertical distribution and its relation to tolerance of desiccation in the New England limpet, *Acmaea testudinalis* has never been reported in the literature. This species, which ranges from Labrador to Connecticut, inhabits the rocky substrata of exposed shores (Miner, 1950; Russell Hunter and Brown, 1964). I have observed that its vertical distribution, in Maine, extends from 1.0–1.5 m above mean low water, into the subtidal area.

The purpose of this study was to determine if *A. testudinalis,* like other limpets, shows intraspecific differences in tolerance of desiccation, those inhabiting the upper levels of the vertical range presumably being most tolerant of desiccation, and those from the subtidal area least tolerant, and whether limpets which are most tolerant exhibit any significant differences in shell shape or size.

Materials and Methods

Limpets from three contrasting habitats were compared in this study: (1) a vertical rock face group (VRFG), (2) a tide pool group (TPG), and (3) a subtidal group (SG). Specimens were collected from two intertidal habitats and one subtidal area along the west shore of Schoodic Peninsula, Hancock County, Maine, (44° 22′N, 68° 04′W) on September 20 and October 16, 1969. Limpets collected from vertical rock faces were found approximately 0.5–1.5 m above mean low water. Specimens collected from tide pools were found near mean low water level, and those obtained subtidally were found approximately 2.0–4.0 m below mean low water.

Collections from vertical rock faces and tide pools were made at low tide by wading, and from the subtidal habitat by SCUBA diving. Approximately 360 specimens were collected from each habitat, each day, except that only 270 specimens could be obtained from the tide pools on October 16. Individuals were removed from the substrate by inserting a thin spatula blade under the foot and prying gently. Individuals with damaged shells were discarded. Specimens were placed in plastic bags containing sea water, placed on ice, and taken to the laboratory within 12 hours.

Two laboratory experiments were conducted in which limpets from each habitat were subjected to periods of desiccation. The specimens collected on September 20 were used in experiment (1). Those collected on October 16 were used in experiment (2) which was identical to experiment (1) except for differences in the number of specimens used, as noted below. A total of 360 specimens from each habitat was placed, 20 specimens per dish, in 18 dry, plastic petri dishes, 14.0 cm in diameter. As a control, 60 specimens from each habitat were covered with artificial sea water at 15° C (the sea water temperature at the collecting site on September 20). The sea water was changed every 24 hours. Five groups, each containing 60 specimens from each habitat, were left uncovered and subjected to desiccation at temperatures ranging from 19.5 to 21° C, the seasonal air temperature. Because only 270 specimens were obtained from the tide pools on October 16, a control group and five other groups, each containing 45 limpets, were used in experiment (2). Fifteen specimens were placed in each petri dish.

Table I

Number and per cent of limpets from vertical rock face, tide pool and subtidal habitats, surviving desiccation at six time periods

Time in hours	Vertical rock face				Tide pool				Subtidal			
	Experiment 1		Experiment 2		Experiment 1		Experiment 2		Experiment 1		Experiment 2	
	Number of 60	%	Number of 60	%	Number of 60	%	Number of 45	%	Number of 60	%	Number of 60	%
0	60	100	39*	98	59	98	45	100	60	100	60	100
12	57	95	60	100	51	85	41	91	60	100	54	90
24	33	55	49	82	7	12	11	24	27	45	14	23
36	8	13	10	17	1	2	0	0	2	3	4	7
48	1	2	4	7	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0	0	0	0	0

* Number surviving of 40.

Each of the five groups was desiccated for a specific time period: 12, 24, 36, 48, or 60 hours. At the end of the desiccation period, each group was covered with 15° C sea water and refrigerated at 15° C for 24 hours, after which it was examined for survivors. The control group was examined at the same time as the group which had been desiccated for 60 hours. The criterion for determining survival was movement of the foot in response to mechanical stimulation. This method was used successfully by Brown (1960) for six species of gastropods.

In experiment 2, five dead specimens were counted in one of the three petri dishes from the VRF control group. The high mortality in this dish was attributed to a build-up of fecal matter around the specimens. This occurred because of a failure to change the sea water in this particular dish for 48 hours. Data obtained from this dish were omitted from the results.

Per cent survival data from the two desiccation experiments were analyzed statistically using the IBM 360 computer at the University of Maine Computing Center in Orono. A randomized block design analysis of variance and a Duncan's multiple range test ($P = 0.05$) were used to determine if survival decreased significantly with increased desiccation time. The two analyses described above were also used to determine whether limpets from one habitat group survived better than those from the other groups during any of the desiccation time periods.

An analysis of variance with interaction and a Duncan's test ($P = 0.05$) were used to determine whether any group showed significantly greater overall survival.

Length, width and height of each shell were measured to the nearest 0.05 mm with dial calipers. Length (L) was designated as the maximum distance from the anterior to the posterior shell margin, width (W), the maximum distance between the lateral shell margins, and height (H), the vertical distance from the ventral margin to the apex of the shell. In addition, the degree of steepness $[S = 2H/(L + W)]$ and the degree of roundness $(R = W/L)$ was determined for each shell. Both equations were used by Orton (1932) for *P. vulgata.*

The shell measurements were analyzed statistically to determine whether shells from the three habitats differed significantly in size and shape. A randomized block design analysis of variance and a Duncan's multiple range test ($P = 0.05$) were used.

Table II

Mean shell characteristics of limpets from vertical rock face, tide pool and subtidal habitats, $P = 0.05$

Habitat group	Mean length (mm)	Mean width (mm)	Mean height (mm)	Mean steepness	Mean roundness
Vertical	18.73	14.24	5.87	3.55	7.68
Tide Pool	14.58	11.03	4.45	3.46	7.60
Subtidal	17.82	14.42	5.37	3.33	8.07

Results

Dessication experiments

As the length of the desiccation period increased, the number of limpets surviving decreased (Table I). Results of the statistical analysis showed that the mean per cent survival of the three habitat groups combined, at each time period, decreased significantly as desiccation time increased. Per cent survival under constantly submerged conditions (0 desiccation time) ranged from 99 to 100%. There was no survival in any of the three groups after 60 hours of desiccation. No specimens from the TPG and SG survived desiccation for 48 hours but a small per cent of the specimens from the VRFG were able to survive desiccation for 48 hours.

The per cent survival of the three groups did not differ significantly at 0, 48, and 60 hours of desiccation. At desiccation periods of 12, 24, and 36 hours, the VRFG had significantly greater per cent survival than the TPG. At 24 hours of desiccation, per cent survival of the VRFG was significantly greater than both TPG and SG. TPG and SG did not differ significantly at any time period.

The mean per cent survival of six time periods, determined for each of the habitat groups as part of the analysis of variance test, was 46.9% for the VRFG, 26.1% for the TPG, and 35.4% for the SG. The per cent survival of the VRFG was significantly greater than both the TPG and SG, and the mean per cent survival of the SG was significantly greater than the TPG. Fifty per cent survival occurred between 12 and 24 hours of desiccation for the TPG and SG, and between 24 and 36 hours for the VRFG.

Shell measurements

Mean length, width, height, steepness, and roundness are listed for each habitat group in Table II. The mean length and width of tide pool specimens were significantly smaller than the mean length and width of the VRFG and SG. There was no significant difference in mean length or mean width between the VRFG and SG. The mean height of tide pool specimens was significantly smaller than the mean height of the VRFG. There was no significant difference in mean height between the VRFG and SG. There were no significant differences in mean steepness or mean roundness between any of the three habitat groups.

Discussion

Several studies of the tolerance of intertidal gastropods to desiccation have shown that such tolerance was correlated with habitat level in the intertidal zone (Colgan, 1910; Gowanloch and Hayes, 1940; Davies, 1969). Individuals of a species inhabiting a high level tolerated desiccation better than low level individuals of that species. This study showed that in the limpet, *A. testudinalis*, tolerance of desiccation was not a characteristic only of specimens from the upper level of its vertical range. Specimens collected from upper level vertical rock faces and from lower level subtidal areas were more tolerant of desiccation than specimens collected from tide pools which were at an intermediate position.

This study showed that tolerance of desiccation in the three habitat groups was related to the size of the organisms. Mean shell sizes of limpets from the vertical rock faces and subtidal habitat were significantly greater than those of limpets from tide pools. The data regarding differences in shell size between limpets from the vertical rock face habitat and those from the tide pools are in agreement with data of Das and Seshappa (1948) who reported that small-sized *P. vulgata* could not tolerate exposure to desiccation, and only large-sized specimens of *P. vulgata* were found under conditions of exposure. In contrast, the results of my study do not support those of Segal (1956) and Shotwell (1950). Segal (1956) found that large and small *Acmaea limatula* were equally tolerant of desiccation. Shotwell (1950) observed that smaller specimens of *Acmaea pelta*, *A. mitra*, and *A. scutum* were more tolerant of desiccation than larger limpets.

Several studies have shown that a steep shell in limpets retards water loss and increases tolerance of desiccation (Orton, 1932; Moore, 1958; Ebling, Sloane, Kitching and Davies, 1962; Jobe, 1968). Davies (1969) found that individuals of *P. vulgata* inhabiting areas of exposure to desiccation had steeper shells, while those in damp areas had flat shells. He concluded that the relatively smaller circumference of the steeper shells resulted in a smaller surface area through which water was lost.

This study showed that in *A. testudinalis*, unlike *P. vulgata*, a steep shell with a small circumference was not characteristic of limpets which were most tolerant of desiccation. Statistical analyses of measurements of shell steepness and roundness showed no significant differences in shell shape in any of the three habitat groups.

Two tentative conclusions can be made regarding the origin of the size distribution pattern in *A. testudinalis*. Both require further investigation to substantiate. The first conclusion is based on an assumption that small limpets in tide pools are young, and larger limpets in the habitats above and below this level are older. The predominance of young limpets in the tide pools would then suggest that the larvae of *A. testudinalis* settle here and migrate outward as they mature. The larvae may settle on rock surfaces which are exposed at low tide, or subtidally, but do not survive in these habitats. Small limpets are herein shown to be least tolerant of desiccation. They may also be more vulnerable to predators such as sea stars which occur subtidally. Blackmore (1969) stated that population pressure caused the outward migration of maturing *P. vulgata* from larval settling areas low in the intertidal zone. Population pressure might also cause the outward migration of *A. testudinalis* from tide pools.

A second explanation of the size distribution pattern in *A. testudinalis* is proposed on the basis of Sutherland's (1970) study of the population dynamics of *A. scabra*. He concluded that size distribution in this species was determined by population density. Low recruitment of limpets in the upper zone, resulting in low population density, permitted the limpets to grow faster and larger at this level. In the lower zone, high recruitment and resultant high population density inhibited growth. A study of the population dynamics of *A. testudinalis* may reveal a similar relationship between size distribution and population density. Perhaps larger numbers of larvae settle in tide pools than above and below this level. Resulting lower population densities in the vertical rock face and subtidal habitats may promote more rapid growth, hence populations of larger limpets at these levels.

The VRFG and SG showed some differences in ability to survive desiccation. Per cent survival of the VRFG was greater than that of the SG at desiccation periods of 12 through 48 hours. (Table I). This difference was consistent although it was significant only at 24 hours. Fifty per cent survival in the VRFG occurred between 24 and 36 hours, while that for the SG occurred earlier, between 12 and 24 hours. Mean per cent survival for the VRFG was significantly greater than the SG. These differences in survival could not be correlated with any differences in size or shape. The factor responsible for the greater degree of survival in the VRFG may have been physiological. Davies (1969) reported that specimens of *P. vulgata* inhabiting the high intertidal zone were better able to limit the rate of body water lost under desiccated conditions than specimens from the low intertidal zone.

The limpets collected from vertical rock faces were found between 0.5 and 1.5 m above mean low water, the upper limit of the intertidal range of this species, where they would be exposed to a maximum of six to eight hours of desiccation on a low tide. Most VRF specimens tested in these experiments were able to survive at least 12 hours. The limpets collected subtidally and from tide pools were never subjected to desiccation in these habitats, yet a high percentage were able to survive 12 hours under experimental conditions.

Thus other factors probably interact with desiccation to limit the distribution of *A. testudinalis*. Other studies have indicated that desiccation alone does not determine the upper limits of an intertidal species (Stephenson, 1942; Test, 1945; Davies, 1969). Temperature, wind and degree of insolation may lower an organism's tolerance of desiccation (Stephenson, 1942). Grazing gastropods, with a strict food preference, may be limited to a lower habitat by their algal food supply which grows at a lower level (Test, 1945). Davies (1969) found that the upper limit of the range of *P. vulgata* was below that which would cause lethal water loss. He suggested that there was a balance between amount of body water lost on exposure and the time required to recover this water when the limpet was submerged during the next high tide. Further study is necessary to determine the factors which may interact with desiccation to limit the vertical distribution of *A. testudinalis*.

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Summary

1. This study examined tolerance of desiccation in the limpet, *A. testudinalis,* collected from two intertidal habitats, vertical rock faces and tide pools, and one subtidal area on Schoodic Peninsula, Hancock County, Maine.

2. The results of two laboratory desiccation experiments showed that subtidal limpets and those from vertical rock faces were more tolerant of desiccation than those from tide pools.

3. Greater tolerance of desiccation was related to the size of the organisms. Mean shell sizes of subtidal limpets and those from vertical rock faces were significantly larger than those of limpets from tide pools.

4. Shell shapes of limpets from the three habitat groups did not differ significantly, which showed that differences in tolerance of desiccation were not related to differences in shell shape.

5. Limpets from vertical rock faces showed a slightly greater tolerance of desiccation than those from the subtidal area.

6. The size distribution of limpets on Schoodic Peninsula suggests either that larvae of this species may settle in tide pools and migrate outward to exposed intertidal and subtidal habitats as they mature, or that population density in each habitat determines the rate and limits of growth of the resident limpets.

7. This species tolerated more desiccation than it would be required to withstand in its natural habitat. Desiccation must act with other factors in determining the upper limit of the distribution of this species.

LITERATURE CITED

Allanson, B. R., 1958. Some aspects of the ecology of the molluscan genus *Siphonaria* in South Africa. *Portug. Acta Biol.* (B), **6**: 179–212.

Blackmore, D. T., 1969. Studies of *Patella vulgata* L.: I. Growth, reproduction, and zonal distribution. *J. Exp. Mar. Biol. Ecol.*, **3**: 200–213.

Broekhuysen, G. J., 1940. A preliminary investigation of the importance of desiccation, temperature and salinity on factors controlling the vertical distribution of certain intertidal marine gastropods in False Bay, South Africa. *Trans. Roy. Soc. S. Afr.*, **28**: 255–292.

Brown, A. C., 1960. Desiccation as a factor influencing the vertical distribution of some South African gastropods from intertidal rocky surfaces. *Portug. Acta Biol.* (B), **8**: 11–23.

Colgan, N., 1910. Notes on the adaptability of certain littoral mollusca. *Irish Natur.*, **19**: 127–133.

Das, S. M., and G. Seshappa, 1948. Biology of *Patella*. *Proc. Zool. Soc. London*, **117**: 653–662.

Davies, P. S., 1965. Environmental acclimation in the limpet *Patella vulgata*. *Nature*, **205**: 924.

Davies, P. S., 1969. Physiological ecology of *Patella*. III. Desiccation effects. *J. Mar. Biol. Ass. U. K.*, **49**: 291–304.

Ebling, F. J., J. F. Sloane, J. A. Kitching and H. M. Davies, 1962. The ecology of Lough Ine. XII. The distribution and characteristics of *Patella* species. *J. Anim. Ecol.*, **31**: 457–470.

Frank, P. W., 1965. The biodemography of an intertidal snail population. *Ecology*, **46**: 831–844.

Glynn, P. W., 1965. Community composition, structure and inter-relationships in the marine intertidal *Endocladia muricata–Belanus glandula* association in Monterey Bay, California. *Beaufortia*, **12**: 1–198.

Gowanloch, J. N., and F. R. Hayes, 1926. Contributions to the study of marine gastropods. I. The physiological factors, behavior, and intertidal life of *Littorina*. *Contrib. Can. Biol. Fish.*, **3**: 133–166.

Jobe, A., 1968. A study of morphological variation in the limpet *Acmaea pelta*. *Veliger*, **11**(suppl.): 69–72.

Lewis, J. R., 1954. Observations on a high level population of limpets. *J. Anim. Ecol.*, **23**: 85–100.

Miner, R. W., 1950. *Field Book of Sea Shore Life*. G. P. Putnam's Sons, New York, 888 pp.

Moore, H. B., 1958. *Marine Ecology*. John Wiley and Sons, Inc., New York, 493 pp.

Orton, J. H., 1932. Studies on the relation between organisms and environment. *Trans. Proc. Liverpool Biol. Soc.*, **46**: 1–16.

Segal, E., 1956. Adaptive differences in water-holding capacity in an intertidal gastropod. *Ecology*, **37**: 174–178.

Shotwell, J., 1950. Distribution of volume and relative linear measurement changes in *Acmaea*, the limpet. *Ecology*, **31**: 51–61.

Russell Hunter, W., and S. C. Brown, 1964. Phylum Mollusca, Introduction, Classification, Glossary and References. Pages 129–152 in R. I. Smith, Ed., *Keys to Marine Invertebrates of the Woods Hole Region*. Spaulding Company, Boston.

Stephenson, T. A., 1942. The causes of the vertical and horizontal distribution of organisms between tide marks in South Africa. In A symposium on intertidal zonation of animals and plants. *Proc. Linnean Soc., London*, Session **154**: 219–232.

Stephenson, T. A., and A. Stephenson, 1949. The universal features of zonation between the tide marks on rocky coasts. *J. Ecol.*, **37**: 289.

Sutherland, J., 1970. Dynamics of high and low populations of the limpet, *Acmaea scabra* (Gould). *Ecol. Monogr.*, **40**: 169–188.

Test, A. R., 1945. Ecology of California *Acmaea*. *Ecology*, **26**: 395–405.

Wilbur, K. M., and C. M. Yonge, 1964. *Physiology of Mollusca, Volume I*. Academic Press, New York, 473 pp.